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LAMB DYSENTERY.

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Lamb Dysentery.

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The disease of lambs which has been named "Lamb Dysentery" was first scientifically investigated by Gaiger(1) in 1919. Although the first scientific records of the disease are those given by Gaiger, it is probable that this condition has existed in localised areas in Britain for many years, *e.g.*, in all probability the disease referred to in a volume published in 1811(2) was lamb dysentery as we know it to-day. On page 191 of this volume the writer says, "the loss of lambs is sometimes very considerable . . . from the 'milk ill,' etc."

The disease is evidently widespread in Britain. From *post-mortem* examination of lambs we have diagnosed its presence in Northumberland, Cumberland and Hampshire in England, in Roxburghshire, Kirkcudbrightshire, Wigtonshire, Peeblesshire, Selkirkshire, Lanarkshire, Argyllshire, Dumfriesshire, Ayrshire, Perthshire and Stirlingshire in Scotland, and in Merionethshire and Montgomeryshire in North Wales. According to Pool(3) it is present in Cornwall, and Hare and Glynn(4) consider that it has probably existed in Africa, the United States and on the Continent of Europe. From private communications we believe it occurs in Peru and other parts of South America, and Jungherr and Welch(5) record the presence of a disease of lambs resembling lamb dysentery in Montana, U.S.A.

The disease as described by most investigators is characterised by the presence of ulcers in the intestinal tract. It may be, however, that although ulceration is a common lesion, many lambs infected with what we believe to be the causal agent show little or no evidence of ulceration. This point will be dealt with more fully later.

It is not proposed to describe the symptoms, lesions, etc., of lamb dysentery. A good description is given by Gaiger and Dalling(6) and others. The purpose of this paper is rather to discuss the disease from an etiological standpoint.

Gaiger's first opinion was that a special strain of *B. coli* was the probable cause of lamb dysentery and this view was held until 1922, when Gaiger and Dalling(7) published the results of further investigations into the disease and described an anærobe which they believed played an important part. Later Gaiger(8) formed the opinion that *B. coli* was the exciting cause of the disease and that soil anærobes, especially *B. welchii* and *vibrion* *septique*, played the rôle of secondary invaders and were responsible for the death of the lamb. This view was also held in 1926 by Pool(3). We have gradually altered our opinion and at the present time we believe that the exciting cause of the disease is a highly toxic anærobe. Our views are based on the following points :—

(*1*) The organism has been isolated from the ulcerated areas of the intestinal tract of diseased lambs.
(*2*) In some cases it has been isolated from tissues, *e.g.*, liver, kidney, blood, etc., of diseased lambs.
(*3*) The organism is not found, as far as our experience goes, in healthy lambs.
(*4*) Soil from infected areas has been shown to harbour this organism.
(*5*) The disease has been artificially reproduced by the use of this organism on healthy young lambs from non-infected areas.
(*6*) Lambs may be protected against lamb dysentery by the use of antigens and antitoxin made from this organism.

THE ORGANISM.

We propose, for the purpose of this paper, to refer to this organism as the " L.D. bacillus." It can, with comparative ease and regularity, be isolated from the intestinal tract of lambs which are suffering from, or which have died from, lamb dysentery. It is also present in the intestinal tract of some lambs which, while showing no actual ulceration, present an acute muco-enteritis, called by farmers and shepherds " milk ill." In many of its characters the organism resembles *B. welchii*, being microscopically indistinguishable. The points of difference from *B. welchii* are (*a*) its toxin ; (*b*) its antitoxin ; (*c*) its action on solid serum ; (*d*) its action on alkaline egg ; (*e*) its action on glycerine.

Toxin.

L.D. bacillus produces a highly potent exotoxin when grown in liquid media. Incubation of the organism for even two hours results in the formation of a toxin of which 0·001 c.c. injected intravenously may kill mice. We have carried out many experiments to find which medium produces the best toxin when inoculated with L.D. bacillus, but have found nothing better than Robertson's peptone meat broth containing cooked meat. The most potent toxin is produced after twelve to fifteen hours' incubation at 37° C. and is toxic for mice, guinea-pigs, rabbits, pigeons and sheep when injected intravenously or intramuscularly.

The special feature of L. D. toxin is its potency compared with *B. welchii* toxin made in exactly similar ways. We have not been able with any of our cultures to produce a *B. welchii* toxin which will kill mice by intravenous injection in doses smaller than about 0·01 c.c., whereas many of the toxins produced by different strains of L.D. bacillus kill mice similarly injected in doses of 0·0001 c.c. The toxin is also noteworthy because of its action when injected intradermally into guinea-pigs or sheep; small doses as low as 0·001 c.c. will produce necrotic lesions. In its liquid state the toxin is unstable; it rapidly loses its potency even when stored on ice for a few days. It can, however, be precipitated by means of ammonium sulphate and this precipitate, dried and powdered, remains stable. We have worked with such a precipitated toxin for about two years; its action has been constant throughout and it is used regularly for testing animals in immunity experiments. The toxin is hæmolytic for rabbit or sheep red cells. The hæmolytic titre of the various strains of L.D. toxin varies considerably, and the hæmolytic power of a toxin bears no relationship to its toxicity. It would appear that L.D. toxin is about 10 to 20 times less hæmolytic than that produced by *B. welchii.*

Antitoxin.

By injecting rabbits, guinea-pigs, sheep or horses with L.D. toxin, or a modification of it, an antitoxin, which will neutralise the action of the toxin, is produced. The most interesting point about L.D. antitoxin is that it completely neutralises both its own toxin and that of *B. welchii.* The reverse, however, does not hold good, *i.e.*, *B. welchii* antitoxin while of high value in protecting against its own toxin does not prevent deaths or reactions in animals by L.D. toxin. Both L.D. and *B. welchii* antitoxin have the power of neutralising the hæmolysins of both L.D. and *B. welchii.*

Solid Serum.

We have now studied 29 strains of the L.D. bacillus isolated from lambs and soil from many parts of the country; all liquify solid serum. Some strains require incubation under strict anærobic conditions for several days before liquefaction of the medium occurs, while others liquefy the serum in one or two days. *B. welchii* fails to liquefy serum even after prolonged incubation. In our laboratory some cultures of *B. welchii* have been stored on solid serum for two years and there is no sign of any liquefaction taking place. It would be feasible to imagine from this character that the culture of the organism is impure, being contaminated with a highly proteolytic type of anærobe. We have made exhaustive examinations and hundreds of platings of the organism without being able to satisfy ourselves that the culture is contaminated. Several well-known anærobe workers have kindly examined a strain of this organism for us. They have all suggested that the culture may be a mixture of two organisms, but so far all attempts to separate the culture into two organisms that provide a satisfactory explanation of the cultural and immunological phenomena have failed. It must be emphasised that every strain has been repeatedly examined by most well-known methods and we have been unable to isolate any other organism; we shall assume, until contrary evidence is available, that the L.D. bacillus which liquefies solid serum is a pure culture.

Alkaline Egg.

The L.D. bacillus rapidly coagulates alkaline egg. All strains studied possess this character though, as in the case of solid serum, the rate at which such coagulation takes place, varies considerably with the strain. We have been unable to show that *B. welchii*, of which 37 strains of human, ovine, equine, porcine and bovine origins, some obtained from European countries, some from laboratories in England and some of which we have isolated ourselves, coagulates alkaline egg.

Glycerine.

Simonds(9) classifies *B. welchii* according to its fermentative action on glycerine and inulin. His groups are :—

Group.	*Glycerine.*	*Inulin.*
1.	+	+
2.	—	—
3.	+	—
4.	—	+

L.D. bacillus fails to ferment glycerine and inulin. All strains agree in this. All strains of *B. welchii* examined by us ferment glycerine, while only a few ferment inulin. No culture of *B. welchii*, in our hands, has failed to ferment glycerine, therefore we cannot agree with the Simonds' classification. A further feature of the L.D. bacillus in connection with its action on glycerine is its failure to form the aldehyde acrolein when grown in glycerine. Humphreys(10) shows that *B. welchii* grown in 2 per cent. glycerine lemco broth produces acrolein as shown by Voisenet's " Glycero-reaction " test. We have confirmed this observation and have failed to demonstrate the formation of this aldehyde by any of the strains of the L.D. bacillus.

Table I has been prepared to show the essential differences between the L. D. bacillus and *B. welchi.*

TABLE I.

	L. D. bacillus.	*B. welchii.*
(*a*) Toxin	Highly potent.	Potent.
(*b*) Antitoxin ...	Neutralises L.D. and *B. welchii.*	Neutralises *B. welchii* but not L.D.
(*c*) Solid serum ...	Liquefies.	Does not liquefy.
(*d*) Alkaline egg ...	Clots rapidly.	Does not clot.
(*e*) Glycerine ...	Does not ferment.	Ferments.
(*f*) Acrolein ...	Does not form.	Forms.

ISOLATION OF THE ORGANISM FROM NATURAL CASES.

It was a matter of some difficulty to isolate this organism in our early investigations, but we have devised methods whereby the organism can be isolated in a very short space of time with a minimum of labour. One method is to spread the sample of intestinal contents on a sterile porcelain plate and the resultant dried material is inoculated into tubes of meat broth. Whenever it is ascertained that *B. welchii*-like organisms are growing (and this can usually be noticed after two or three hours' incubation) subcultures are made into the same medium and this method of rapid subcultivation is carried out until a microscopically pure-looking culture is grown. Plating on solid material is now carried out, either on blood agar or on a medium with a basis of tryptic digest agar to which is added a mixture of rabbit serum and hæmolysed rabbit red cells (a method devised by Mason and as yet unpublished) and after 24 hours' incubation under good anærobic conditions it is usually possible to obtain large colonies of the L.D. bacillus in pure culture. It is not always easy to obtain cultures of the organism from other tissues ; Gaiger and Dalling(7) record that the organism has been isolated from various tissues in twelve cases. It is probable that all these organisms were not of the L. D. variety, but that some were typical *B. welchii*, for, when that work was carried out, the differences between L.D. bacillus and *B. welchii* were not known. Recently, however, we have been able to isolate the typical L.D. bacillus from the heart blood of six out of eighty lambs which had died of typical lamb dysentery. It would appear that it is only under exceptional conditions that the L.D. bacillus penetrates from the intestine before death. Of the many *post-mortem* examinations carried out during the past four years, in twelve only have we been able to isolate the L.D. bacillus from tissues, *e.g.*, liver, kidney, heart blood, etc., of lambs which were killed while suffering from the disease. Under such circumstances the organism is isolated in a pure state. In the intestinal contents of lambs acutely affected with lamb dysentery the organism is abundant and there is no necessity to culture actual ulcers as was done in the past; in fact, we have been able to isolate organisms from lambs which have shown no evidence of ulceration but which have been affected with a muco-enteritis termed " milk ill " by shepherds and farmers. Gaiger(8) states that he isolated *vibrion septique* from the ulcers of lamb dysentery, and Pool(3) mentions that *vibrion septique*, etc., have been isolated from the tissues of affected lambs. Although we have used all the resources at our command in our search for *vibrion septique*, we have failed to find this organism in the intestinal contents, ulcers or tissues of lambs acutely infected with lamb dysentery. Lambs in the chronic or sub-acute stage of the disease and which have been infected for some days or weeks have yielded cultures of *vibrion septique* from partly-healed ulcers, but not from other tissues.

During the course of our experiments we have had opportunity of examining the intestinal contents of healthy lambs killed for such a purpose and also of lambs which have died from diseases other than lamb dysentery in areas where lamb dysentery did not exist. From none of these lambs (16 examined) have we been able to isolate the L.D. bacillus. Thus we are forced to the conclusion that the L.D. bacillus is present only in lambs suffering from the disease known as lamb dysentery and that this condition, although usually associated with the presence of ulcers in the intestine, may exist where no ulceration can be demonstrated. We are of opinion that much of the so-called " milk ill " especially in the lamb dysentery areas, is a phase of lamb dysentery, and from some statistical evidence which we are now collecting, it appears that some of the so-called " grass ill " cases among lambs up to four weeks old are really cases of lamb dysentery.

The Organism in the Soil.

During the past four years six samples of soil have been collected from various farms on which lambs die from lamb dysentery. Four samples have also been collected from areas where lamb dysentery does not exist. The L.D. bacillus was isolated from four of the former samples, but we have been unable to obtain any evidence of its existence in soil from land on which the disease is unknown.

THE ARTIFICIAL REPRODUCTION OF THE DISEASE.

The earliest records of the artificial reproduction of lamb dysentery are by Gaiger(1) who claims to have set up in young rabbits a fatal enteritis with lesions in the bowel resembling those seen in lambs. Gaiger and Dalling(6) in 1921 record that the subcutaneous injection of 5 c.c. of a culture of the *B. coli* organism killed a lamb in 48 hours and though the lamb showed no diarrhœa or dysentery, " on *post-mortem* examination inflamed and ulcerated lesions were found in the bowels indistinguishable from the natural disease." In the same paper they record that a lamb fed with fæces from a ewe, " died presenting clinical symptoms of the disease." This work, however, was carried out on an infected farm and it is possible that the infection was not produced by the experimental procedure. In 1922 Gaiger and Dalling(7) published further results on the artificial reproduction of lamb dysentery. In their experiments they fed lambs with a mixture of cultures of B. *coli* of lamb dysentery origin and L.D. bacillus and produced typical lamb dysentery, while the feeding of either organism alone produced no ill-effect. In 1924 Dalling (11) successfully repeated this work in Kent. In 1925 Dalling, Allen and Mason(12) published results of experiments confirming the results of feeding lambs with *B. coli* and L.D. bacillus. In the same paper they showed that the intravenous inoculation of ground up ulcers produced lamb dysentery, while the intravenous injections of scrapings from the mucous membrane of a normal lamb's intestine produced no harmful results in a normal lamb. In 1926 we first showed that the L.D. organism alone was capable of setting up lamb dysentery. The disease was reproduced by the feeding of culture and by its intravenous injection. Some of the most typical cases of artificially produced lamb dysentery have been set up by the intravenous injection of small amounts of the lamb dysentery bacillus culture. The histological picture of the ulcerations produced in the artificially infected lambs was indistinguishable from that of natural cases. Sections of ulcers, etc., were examined by a well-known histologist whose opinion is that quoted. A probable explanation of the successful reproduction of lamb dysentery by L.D. bacillus alone is that in 1926 the culture of L.D. bacillus used was much more virulent than in former years, due to its virulence being enhanced by passage through normal lambs immediately before experimentation. We have not had the opportunity of repeating these experiments, but we are satisfied that we can reproduce lamb dysentery artificially by the feeding or by the intravenous injection of a culture of highly virulent L.D. bacillus to young healthy lambs, preferably those which have not had an opportunity of sucking their mothers before being treated. There is no doubt that the culture was responsible for the production of the symptoms and lesions of lamb dysentery because a specific antiserum prevented the action of the organism, while normal horse serum did not influence its action. Attempts were made to produce lamb dysentery by injecting *vibrion septique* cultures intravenously; the results were entirely negative although the culture had been passaged in lambs before use.

During the course of our experiments we have had several failures, but in view of the opinion we have formed that ulceration is not necessarily the characteristic symptom of lamb dysentery, some of our experiments really gave positive results although we counted them negative. We believed at that time that ulceration was the diagnostic symptom of lamb dysentery and we have only recorded our attempts as successful when actual ulceration was produced. We felt with Pool(3) that "an observer would be very rash to draw any conclusions from cases in which the typical ulcers were absent," and hence all our recorded work referring to the successful reproduction of lamb dysentery artificially means that actual ulcers were present in the intestine and could be demonstrated macroscopically.

Pool(3) has recorded in a small note that he has been able to reproduce lamb dysentery by feeding normal young lambs with the contents of the intestinal tract of diseased lambs, thus proving that the disease is contagious. In 1922 Gaiger and Dalling(7) showed similar results, two lambs being so infected, one dying on the fifth and one on the sixth day after feeding. We(13) also have shown that the intravenous injection of such material produces typical lamb dysentery.

IMMUNISATION AGAINST LAMB DYSENTERY.

It was early realised that any treatment to be adopted in the prevention of lamb dysentery in lambs must be applied to the lamb within a few hours after birth or *via* the mother ewe. We are in the position to-day to state definitely that lamb dysentery can be prevented either by passively immunising the lamb with an antitoxin made from the L.D. bacillus or by actively immunising the mother ewe with an antigen made from this organism.

Use of L.D. Antitoxin in Lambs.

When it was believed that *B. coli* played the all-important part in the causation of lamb dysentery, a *B. coli* antiserum made in horses was tried in the field. It was deduced from one year's experiments that hopeful results had been got(6), but the following year the apparent good results could not be repeated and hence the method was abandoned(7). In 1924 we produced an antitoxin by injecting a horse with the toxin of a strain of L.D. bacillus. It was mixed with an equal volume of *B. coli* antiserum and young lambs were inoculated with 10 c.c. of the mixture, control uninoculated lambs being left on the same farms. From our recent experiments we know that the antitoxin prepared in 1924 was of relatively low value compared with that used in later seasons, nevertheless, in the limited number of experiments already recorded(11), encouraging results followed its use : thus, for example, in one district, of a total of 390 lambs inoculated, 30 or 7·7 per cent. died of lamb dysentery, while of 75 controls, 30 or 40·0 per cent. died. On one farm in the same district 30 lambs were injected each week for three weeks ; of these, two died, while of 30 control lambs, 14 died. In 1925 the same serum was used and the results of experiments carried out were that 64 of 720 inoculated lambs (8·9 per cent.) and 139 of 365 controls (38·8 per cent.) died(12). Antitoxin of higher protective value was now made and the results of experiments in 1926 have already been recorded(13) ; of 456 lambs inoculated with the mixed serum, 9 or 1·9 per cent. died, while of 425 uninoculated, 106 or 22·6 per cent. died. These inoculations were carried out on five different farms and all the lambs which died were subjected to *post-mortem* examination. In addition to these controlled experiments, over a thousand lambs were injected on ten farms where no controls were left and only two died of lamb dysentery. During 1926 we had come to the conclusion that the main factor in the causation of lamb dysentery was the L.D. bacillus and hence in 1927 a series of experiments, in which 5 c.c. of a pure L.D. antitoxin was administered to the lamb soon after birth, was carried out. On fifteen farms in Northumberland, Scotland, Wales and Hampshire, 1,122 lambs received serum ; of these, 5 or 0·44 per cent. died of lamb dysentery, while of 1,241 uninoculated lambs on the same farms, 213 or 17·16 per cent. died(15).

Further experiments have been carried out during the past lambing season ; pure L.D. antitoxin of high value has been used. The results are about to be published and amply confirm the results of previous years. Some examples may be noted here. In North Wales, Dr. R. F. Montgomerie, Bangor, carried out experiments in which 545 lambs on thirteen farms were inoculated soon after birth without any loss from lamb dysentery, while of

380 uninoculated lambs on the same farms and born at the same time as those inoculated, 84 or 22·1 per cent. died. On a farm in Stirlingshire, Mr. G. C. Inglis, M.R.C.V.S., Alloa, carried out an experiment in which, of 618 lambs born 320 received serum, while 298 were not inoculated (alternate lambs born were treated). Four or 1·25 per cent. of the inoculated and 61 or 20·47 per cent. of the untreated lambs died of lamb dysentery. On a farm in Perthshire, 2 out of every 3 lambs born received serum while every third lamb was left as a control. In this way 428 lambs were inoculated, while 202 received no serum. Of the former, 2 or about 0·5 per cent. died of lamb dysentery, while the death rate among the others was 56 or 12·8 per cent.

These few examples of the results of the experimental work in the 1928 lambing season will suffice to show that the results of former years have been amply confirmed. From this work alone we believe that the L.D. bacillus is the important factor in the causation of lamb dysentery and that by the use of its antitoxin on young lambs the disease can be prevented to a great extent.

During our study of the making of L.D. antitoxin we have largely made use of the knowledge obtained from the various researches carried out by our colleagues. At the present time we are able to produce a very high grade antitoxin.

Protection of the Lamb by inoculation of the Ewe.

It has been shown by several workers, *e.g.*, Ehrlich(16), Sudmersen and Glenny(17), Nathan, Larrier and Grasset(18), etc., that females, for example guinea-pigs and mice, when actively immunised will transfer a passive immunity to their offspring. Taking advantage of this knowledge we have carried out many experiments and have proved that lambs born from ewes which have been actively immunised with antigens made from L.D. bacillus resist lamb dysentery infection and possess L.D. antitoxin. This has been proved in two main ways : (*a*) in the field by comparing the death rate from lamb dysentery among lambs born from inoculated ewes with that among those born from uninoculated ewes on the same land ; (*b*) by actually testing the serum of inoculated ewes and their lambs for the presence of L.D. antitoxin. A further method, carried out on a very small scale during the past season, was the intradermic testing of ewes and lambs with L.D. toxin.

Inoculation of Ewes.

The earliest experiments were carried out in 1922 when Gaiger and Dalling(7) used a sero-vaccine made from *B. coli* of lamb dysentery origin. It was proved at this time that pregnant ewes could be inoculated with a foreign material without its harming them and without causing abortion. The result of this work was disappointing and, in the light of our present-day knowledge, it is not surprising that no good results accrued from these inoculations. In the spring of 1925 an antigen made from L.D. bacillus was used in the inoculation of pregnant ewes(12). A toxin of L.D. bacillus was carefully titrated with antitoxin and a neutral mixture made. An equal volume of a killed saline suspension of the lamb dysentery strain of *B. coli* was added and ewes were injected once or twice during pregnancy. Experiments were carried out by Mr. H. McD. Paul, M.R.C.V.S., Alnwick, on six farms, on four of which carefully controlled work was done and lambs dying from all causes were examined. The result was that of 514 lambs born from inoculated ewes, 12 or 2·4 per cent. died of lamb dysentery, whereas of 1,627 control lambs, 393 or 24·1 per cent. died. This result so encouraged us that during 1925-26, with Mr. Paul's collaboration, a very extensive experiment was arranged. On the small scale experiment of 1925 no harmful results from the use of the vaccine were noted, so for the 1925-26 experiments it was arranged to inoculate ewes at different times of the year and to give a varying number of inoculations. Unfortunately, in the spring of 1926 some "break downs" occurred following the use of the vaccine, in that on some farms there was a small percentage of deaths among ewes soon after inoculation. The result of this was that many of the ewes which should have had two inoculations in the spring were given one only. However, we were able to obtain some valuable information as a result of the season's work. The results are fully recorded elsewhere(13), the sum total being that inoculations of the ewes carried out once in the autumn before pregnancy and once in the spring near lambing gave excellent results, in that of 5,760 lambs born from ewes so inoculated, 3·06 per cent. died of lamb dysentery, while of 2,509 lambs born from uninoculated ewes, 16·06 per cent. died. It was decided then that in future work inoculations on these lines should be carried out. During 1926-27 further experiments(15) were made on the same lines with a vaccine similarly prepared. In carefully controlled work in Scotland and Wales on thirteen farms, 4,140 lambs were born from inoculated ewes; of these, 36 or 0·87 per cent. died, while of 2,213 lambs from control ewes, 178 or 8·04 per cent. died. Experiments were also carried out to show the value of L.D. antigens alone without the addition of *B. coli* suspensions. It was possible to arrange that some ewes were inoculated in the autumn and again in the spring and others twice in the spring. In the first group 466 lambs were born from inoculated ewes and 5 or 1·08 per cent. died of lamb dysentery, while 105 lambs were born from control ewes, and of these, 14 or 13·33 per cent. died. Of the second group, 1,934 lambs were born ; 42 or 2·17 per cent. died, while of 187 lambs born from uninoculated ewes, 10 or 5·34 per cent. died. From such work we had some evidence that L. D. antigen alone was effective in producing immunity in the lamb *via* the mother. During the past season a large experiment was carried out in Scotland to test thoroughly whether L.D. antigen alone would protect lambs *via* the mother. The work was controlled on all farms by the use of vaccine composed of L.D. and *B. coli* antigens and by leaving some ewes uninoculated. Briefly, the results of these experiments, which are about to be published, are that 2,065 lambs were born from ewes inoculated with the mixed vaccine ; 14 or 0·67 per cent. died of lamb dysentery. 741 lambs were born from ewes inoculated with a toxin-antitoxin mixture made from L.D. bacillus ; 8 or 1·07 per cent. died. 1,157 lambs were born from ewes inoculated with an " anaculture " made from L.D. bacillus ; of these, 13 or 1·12 per cent. died. 2,530 lambs were born from uninoculated ewes on the same farms ; of these, 330 or 13·09 per cent. died of lamb dysentery.

It is evident, then, that antigens made from L.D. bacillus, injected into ewes as above described, have practically the same value in preventing lamb dysentery in the offspring of these ewes as has a vaccine composed of a mixture of antigens made from L.D. bacillus and *B. coli.*

From these experiments alone there would appear to be reasonable grounds for assuming that L.D. bacillus is the chief, or possibly the only, cause of lamb dysentery in lambs.

The Testing of L.D. Antigen.

Until very recently the L. D. antigen used in ewes was a toxin-antitoxin mixture. It is essential that the mixture be absolutely neutral, because our experience shows that if it is even slightly under-neutralised the free toxin may kill sheep, and if over-neutralised the degree of immunity produced falls short of the standard expected. Guinea-pigs are injected twice at a fortnight's interval and are tested a fortnight later for immunity by injecting various doses of toxin intravenously.

It came as a surprise to us when we found that a toxin-antitoxin mixture may retain its antigenic value only for a comparatively short period. This was brought home to us because of some unexpectedly poor results in Northumberland in 1927(15). The batch of vaccine used as a second inoculation on some sheep had been stored for some months and was not tested immediately before use, thus, while the batch was of high antigenic value when made and used as a first inoculation, when given as a second inoculation six months later, it had lost at least 50 per cent.

Recently, in the field, we have used "anaculture" of L.D. bacillus made by the addition of formalin to a culture under certain conditions. The tests as to antigenic value are carried out as above. We believe that this "anaculture" produces as good immunity as, or possibly better than, a toxin-antitoxin mixture.

Testing of Serum of Ewes and Lambs.

In addition to showing that L.D. antigen protects against lamb dysentery in the field, we have bled many ewes and their lambs, and tested their serum for L.D. antitoxin content. The method of testing consists in the mixing of serum and toxin and its injection into mice intravenously or into guinea-pigs intradermally and noting results. We have shown that a fixed amount of a special dry test toxin forms a sure killing and a sure reacting dose. Before carrying out tests with the serum of vaccinated ewes and their lambs we thoroughly tested serum from normal sheep for its L.D. antitoxin content. The following groups of sheep were tested :—

(*a*) Ten pregnant ewes brought from an unaffected farm in Kent to the laboratory fields were lambed there. Samples of blood were drawn before and after lambing, and on no occasion could any antitoxin be demonstrated.

(*b*) Ten ewes, four to six weeks after lambing on a non-infected farm in Scotland, were bled ; no antitoxin was found.

(*c*) Five lambed ewes on a non-infected farm in an infected district in Northumberland were tested ; antitoxin was present in all samples of their serum. It is of interest to note that the serum samples were obtained in 1926, at which time lamb dysentery had not made its appearance on the farm, but in the spring of 1927 we were able to diagnose the disease in many lambs.

(*d*) Five young virgin sheep from Kent were killed for food and samples of serum were obtained from the local abattoir ; no antitoxin was demonstrated.

(*e*) From 104 lambed ewes on infected farms in Scotland and England samples of serum were obtained ; in 21 samples antitoxin was shown to be present.

Serum from Injected Ewes.

Examples of experiments on testing serum from inoculated sheep carried out are :—(*a*) During the past three years 93 samples of serum from inoculated ewes on infected farms have been tested. At the same time 104 samples from non-inoculated sheep kept under the same conditions were tested. The results of these tests show that antitoxin was demonstrated in 53 of the 93 samples (57·8 per cent.) from the inoculated sheep, whereas in only 21 of the 104 samples (20·1 per cent.) from uninoculated sheep was antitoxin detectable. The figure 57·8 per cent. is not a true indication of the production of antitoxin in these ewes for they were not bled until four or six weeks after lambing, *i.e.*, probably eight to ten weeks after their last injection. (*b*) On two infected farms experiments were carried out and these show that one injection of a toxin-antitoxin mixture produces some antitoxin in injected sheep and that a second injection raises the antitoxin value still further. Sixteen sheep which had received one injection three or four weeks before lambing were bled four to six weeks after lambing ; 15 non-inoculated sheep on the same farms were also bled. Samples of serum marked " A " were so obtained. Immediately after bleeding the 31 sheep received an injection of toxin-antitoxin mixture ; ten days later samples of serum marked " B " were obtained. The testing of these samples showed the presence of antitoxin as follows :—

Samples "A."

Inoculated sheep ...	8 out of 16, *i.e.*, 50·0 per cent.
Uninoculated sheep	2 out of 15, *i.e.*, 13·3 per cent.

Samples B.

Inoculated sheep ...	15 out of 16, *i.e.*, 93·8 per cent.
Uninoculated sheep	5 out of 16, *i.e.*, 33·3 per cent.

(*c*) Six pregnant sheep were brought from a clean farm to infected land and were bled after arrival and samples "A" serum obtained. On the same day each received an inoculation of vaccine and ten days later were bled and samples " B " obtained. On this day they were again injected and ten days later samples " C " were got. The serum was tested by the already described methods and the results were that no antitoxin was demonstrated in samples " A," in samples " B " one sheep showed antitoxin, whereas samples " C " of all sheep showed sufficient antitoxin to neutralise one mouse-killing dose of toxin, and in four there was sufficient to neutralise at least three sure killing doses.

Serum from Lambs.

We have studied the question of the transference of this immunity from the mother ewe to the lamb. Although some field work has been done showing that lambs from immune ewes possess antitoxin, more accurate results have been got from work done on ewes and lambs at the laboratory. Eight pregnant sheep were used for this experiment. They were bled and then injected. They were again injected before lambing and were bled several times after lambing. The lambs were bled soon after birth and again in about a week's time. Table II shows that at the time of bleeding, the lambs' serum contained about the same amount of L.D. antitoxin as that of the mother.

TABLE II.

Comparison Mother Ewe and Lamb serum—presence of antitoxin.

No. Ewe and Lambs.		Lamb Serum (day of birth).	Lamb Serum (7-9 days old).	Ewe Serum (day of birth of Lambs).
41	...	Nil.	—	Nil.
42	...	(*a*) No P.*	—	
		(*b*) P.$_3$	P.$_3$	P.$_2$
		(*c*) P.$_3$	P.$_3$	
43	...	(*a*) Nil.	—	P.$_2$
		(*b*) Nil.	—	
44	...	(*a*) Nil.	—	Nil.
		(*b*) Nil.	—	
45	...	(*a*) P.$_3$	—	P.$_3$
		(*b*) P.$_3$	P.$_3$	
46	...	(*a*) Nil.	Nil.	Nil.
		(*b*) Nil.	Nil.	
47	...	(*a*) Nil.	P.$_2$	P.$_2$
		(*b*) Nil.	P.$_2$	
48	...	P.$_2$	P $_2$	P.$_1$

Test done in mice i.v. 0·2 c.c. serum mixed with one or more M.L.D.s. toxin stood for about one hour before injecting.

* This lamb died soon after birth.

Nil=No protection—all mice died.

P.$_3$, etc. = Protected mice against 3 M.L.D.s. toxin, etc.

During the 1928 season it was possible to test inoculated and control sheep in the field intradermally. For this purpose the under-surface of the tail was used. Ewes and lambs were tested soon after the lamb was born ; samples of blood were drawn at the same time and tested for antitoxin content as already described. The field intradermic and the laboratory results agree very closely and show that to a large extent ewes which possess antitoxin give birth to lambs which, at the period of testing, also possess antitoxin. Detailed results are being published.

From these various experiments it may be deduced that :—

(*a*) Sheep running on non-infected land show no evidence of having been infected with L.D. bacillus in that they possess no detectable antitoxin.

(*b*) Some sheep on infected land show evidence of L.D. antitoxin which indicates that at some period of their lives they have been infected with L.D. bacillus, *e.g.*, as lambs or in adult life through wounds, etc.

(*c*) The injection of L.D. antigen causes the production of, or the increasing of, the amount of L. D. antitoxin in ewes.

(*d*) Ewes transmit antitoxin to their offspring because the serum of immunised ewes and that of their lambs have about the same antitoxin content.

Ratner and his colleagues(19) show that in all probability most of the immunity transferred from mother to offspring in ruminants is *via* the colostrum. If such work is accepted, then, in all probability, the first milk sucked by the lamb contains sufficient antibody to protect it and it is this antitoxin we have demonstrated in lambs' serum. At the present moment we are carrying out an experiment which should settle this point. At the time of writing two inoculated ewes have not yet lambed, but are secreting colostral milk. Samples of their colostrum have been shown to contain a large quantity of L.D. antibodies. We hope to be in the position at the N.V.M.A. Congress in September to give a full account of this work and to show whether the lamb from an inoculated ewe is actually born immune or whether it receives its immunity by way of the mother's milk.

Summary and Conclusions.

(*1*) Lamb dysentery is caused by the toxin of a specific organism which differs from typical *B. welchii* in (*a*) its toxin is serologically different, and (*b*) culturally there are a number of important differences.

(*2*) Although ulceration of the bowel is commonly associated with lamb dysentery, the organism can be isolated from lambs dying of " milk ill " in which no ulceration is present.

(*3*) The conclusion that the organism is the cause of lamb dysentery is based on:—

(*a*) Its constant presence in affected lambs and its absence in normal lambs and those dying from diseases other than lamb dysentery.

(*b*) Its presence in the soil of infected land and its absence from other soils.

(*c*) Its ability to reproduce the disease when fed to or injected into normal lambs.

(*d*) The ability of its antitoxin to prevent lamb dysentery in lambs on infected farms.

(*e*) The protection of lambs from lamb dysentery by the inoculation of ewes with antigen made from the organism, antibodies being transferred from the ewe to the lamb probably mainly in the milk.

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